

Amendment  
U.S. Patent Application No. 09/536,351

**REMARKS**

Reconsideration and continued examination of the above-identified application are respectfully requested.

The amendment to the claims is editorial in nature and/or further defines what applicants regard as their invention. Full support for the amendment exists in the application as originally filed including, but not limited to, the claims as originally filed.

Claims 13 and 20-22 have been cancelled. Claims 1, 3, and 14 have been amended to include the phrase, "and contains substantially no water." Support for this appears at page 5, lines 27-31. Claims 6, 7, and 10 have been amended to recite the basis for the stated weight percentages, i.e., the weight of the pesticide formulation. Support for this occurs at page 6, lines 8-12, which clearly refers to the weight percentages as being based on the weight of the pesticide formulation, which may also be considered a concentrate. A minor typographical error in claim 10 has been corrected. Accordingly, no questions of new matter should arise, and entry of the amendment is respectfully requested.

At page 2 of the Office Action, the Examiner rejects claims 1-8, 10-13, and 17-24 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject material that applicants regard as the invention. More specifically, the Examiner states that the phrase "storage stable" is relative while the word "substantially" is indefinite. For the following reasons, this rejection is respectfully traversed.

The meaning of the phrase "storage stable" is clear to one skilled in the art as reflected by one or more references cited by the Examiner. Furthermore, the present application describes this term. For instance, at page 7, lines 24-30, it is reported that the subject composition is storage

## Amendment

U.S. Patent Application No. 09/536,351

stable for 1 year in a sealed container at 25°C. Therefore, the meaning of the phrase "storage stable" is clear to one skilled in the art. Accordingly, this rejection should be withdrawn.

Insofar as the Examiner's remarks about the meaning of the word "substantially," this term is used in claim 2 with respect to the lack of water present. This term is also used in claims 17-19 with respect to the lack of solvent present. With respect to the lack of water, examples are provided at page 5, lines 27-31 (e.g. preferably less than about 2% by wt.). With respect to the lack of organic solvent, as shown in the examples, no organic solvent is intentionally added. The advantages of not using organic solvents are illustrated at page 7, lines 31-33. The meaning of this term is also clear to one of ordinary skill in the relevant art, especially in light of the portions of the specification quoted. Accordingly, this rejection should be withdrawn.

Also at page 2 of the Office Action, the Examiner asserts that claims 6, 7, and 10 are ambiguous, on the grounds that no basis is given for the stated weight percentages. With respect to this part of the rejection, it is clear that the present application indicates that the weight percentages are based on the weight of the pesticide formulation. For instance, the passage at page 6, lines 8-12 clearly refers to the weight percentages based on the weight of the pesticide formulation. This is also true with respect to the other components such as the vegetable oil and the non-ionic surfactant as shown at page 7 of the present application. Accordingly, although the applicants do not believe that one of ordinary skill in the art would fail to clearly understand the present wording, claims 6, 7, and 10 have been amended to recite that the weight percentages are based on the weight percentage of the pesticide formulation. Accordingly, this rejection should be withdrawn.

## Amendment

U.S. Patent Application No. 09/536,351

Also at page 2 of the Office Action, the Examiner rejects claims 1-3, 5, 14, 17, and 19-21 under 35 U.S.C. §102(b) as being anticipated by Kleeberg (EP 0 579 624). More specifically, the Examiner asserts that claim 1 of Kleeberg shows the present invention, and that dilution is shown in claims 10 and 17 of Kleeberg. For the following reasons, this rejection is respectfully traversed.

Kleeberg is substantially different from the claimed invention in several ways. First, claim 1 of the cited reference shows two products obtained by two different processes: (1) a precipitate of an azadirachtin rich compound; and (2) an azadirachtin solution with a surfactant solvent. Neither of these products or processes shows a pesticide formulation that contains at least one vegetable oil, such as sesame oil, as in the claimed invention. Second, although Kleeberg refers to a material having a long storage life, that material is a powdery concentrate. The claimed invention is not merely a precipitate that contains azadirachtin. The claimed invention is a multi-component pesticide formulation. Additionally, unlike the claimed invention, the actual storage life of the material is not specified. As for the material dissolved in surfactant, it can only be stored for "several months." Therefore, Kleeberg does not show a "storage stable" pesticide formulation, as that term is used in the claimed invention.

As for the specifics of the other identified claims of Kleeberg, claim 10 refers to the ratios of solvent to water (1:10) in the previous claims. Claim 17 refers to the case in which the azadirachtin-rich surfactant phase is diluted with water in a specified ratio. Both of these claims appear to refer to a step in the process of producing the products of claim 1, i.e., a precipitate or a surfactant/azadirachtin solution. In other words, the details of the extraction step used to remove organic compounds from the raw plant material are shown. This is only one step used in producing the products of claim 1. The identified claims do not recite a subsequent step

## Amendment

U.S. Patent Application No. 09/536,351

involving the production of a multi-component pesticide formulation with the components specified in the claims, or the application of such a formulation to plant life. Therefore, these claims do not seem pertinent to the claimed invention..

Accordingly, in light of all the above, this rejection should be withdrawn.

At page 3 of the Office Action, the Examiner rejects claims 1-7, 14-17, and 19-21 under 35 U.S.C. §102(b) as being anticipated by the 1997 article by Dimetry et al. More specifically, the Examiner asserts that the cited reference shows an azadirachtin compound with a surfactant and sesame oil at designated percentages and dilution with water. For the following reasons, this rejection is respectfully traversed.

As for claims 1-7, the claimed invention is a pesticide formulation comprising at least one vegetable oil, at least one surfactant, and azadirachtin or a neem seed extract, wherein the formulation is storage stable and contains substantially no water. The Dimetry et al. formulation and the claimed invention differ on the grounds that Dimetry et al. only shows a formulation diluted with water, as can be seen from column 1 at page 396. However, the claimed invention as recited in claims 1 and 3, contains substantially no water. The lack of water is an important feature of the claimed invention, since the presence of water is known to degrade azadirachtin, as is set forth at page 5, lines 19-23 of the application, and further explained in the enclosed excerpt from the Pesticide Research Journal, dated June 1999.

As for claims 14-17, claim 14 recites that substantially no water is present. Claims 15 and 16 are both ultimately dependent on claim 14, and claim 17 depends on claim 1. Therefore, all the identified claims recite that substantially no water is present, and therefore are different from the cited reference for the reason noted above.

## Amendment

U.S. Patent Application No. 09/536,351

As for claims 19-21, claim 19 depends on claim 14, which recites that substantially no water is present. Therefore, this claim is also distinguishable from the cited reference. Claims 20-21 have been cancelled.

Finally, the cited reference does not recite that the formulation is storage stable, which is recited in the claims as a feature of the current invention. This lack of storage stability in the cited reference is logical given that Dimetry et al. teaches a formulation containing water, and, as previously noted, azadirachtin is unstable in the presence of water and other solvents. Accordingly, in light of all the above, this rejection should be withdrawn.

Also at page 3 of the Office Action, the Examiner rejects claims 1-5, 14, 15, 17, and 19-21 under 35 U.S.C. §102(b) as being anticipated by the 1983 article by Schauer. More specifically, the Examiner asserts that the cited reference shows a neem seed extract with synergistic sesame oil, lethicin surfactant, and glycerol, and shows a formulation having insecticide properties, as recited at page 143 of the cited reference. For the following reasons, this rejection is respectfully traversed.

There are significant differences between the cited reference and the claimed invention. First, as set forth at page 142, the formulations were prepared by dissolving a tertiary-methyl-butyl ether extract ("MTB") in methanol and distilled water, and then adding aqueous solutions of various additives, such as glycerol, sesame oil, different lethicins, or other additives. The formulation described in Schauer has been diluted with water prior to the addition of sesame oil. Claims 1, 3, and 14 recite that substantially no water is present. Clearly, Schauer is quite different from the claimed invention. Further, new Neem seeds contain a variety of compounds. It is not clear what active components would be in an MTB extract of a neem seed and whether

## Amendment

U.S. Patent Application No. 09/536,351

azadirachtin would be a significant component. It is worthwhile to note that the cited reference does not even directly mention azadirachtin.

In addition, the cited article does not recite improved storage stability, which is a feature recited in the claims of the present invention. This is not surprising since Schauer used water in forming the composition.

Also, the cited article characterizes sesame oil and lethicins as "carriers" at the bottom of page 142. Lethicin is considered an ionic substance which degrades active ingredients. Certain claims of the present application recite non-ionic surfactants.

Accordingly, in light of all the above, this rejection should be withdrawn.

Also at page 3 of the Office Action, the Examiner rejects claims 1-24 under 35 U.S.C. §103(a) as being unpatentable over Larson (U.S. Patent No. 4,556,562) in view of Kleeberg, Dimetry, and Ujihara (U.S. Patent No. 6,034,128). The Examiner asserts that Larson shows a storage stable azadirachtin with surfactant, neem oil, and substantially no water or solvent, that is stable for up to 2 years. The Examiner concludes that it would have been obvious for a person of ordinary skill in the art to use Larson's composition with the A and B constituents shown by Kleeberg, and with the adjuvant shown by Dimetry, to produce the claimed invention. For the following reasons, this rejection is respectfully traversed.

As an introductory comment, the applicants note that the significance of the Ujihara reference to the present rejection is unclear, because the Examiner does not specifically explain the relevance of this reference in the rejection and it is not apparent from an examination of the reference itself. Ujihara relates to a compound having use as an insecticide, but it does not seem

## Amendment

U.S. Patent Application No. 09/536,351

relate to azadirachtin or a component of neem seed oil. If this reference is material to the rejection, then further clarification from the Examiner is needed and is respectfully requested.

Additionally, although azadirachtin has at least two forms, A and B, this fact does not appear to be directly mentioned in the Kleeberg reference. Therefore, the cited reference does not illustrate the two forms of azadirachtin recited in claim 8, and the significance of the Kleeberg reference is also unclear. If the applicants have misconceived the nature of this rejection, then further clarification from the Examiner is respectfully requested.

As for the remaining aspects of the rejection, the applicants do not believe that the cited references alone or combined teach or suggest the claimed invention, for several reasons. First, Larson already relates to a storage stable composition and explains how that storage stable capability would be achieved. Accordingly, one skilled in the art would not have a reason (i.e., no motivation) to look to any other publication since Larson already provides a method to achieve storage stability. Therefore, one of ordinary skill in the art would not seek to combine the teachings of Larson with any other references to solve the stability problem, since Larson already provides a solution to that problem.

Second, as can be verified from the abstract, the solution provided in Larson is quite different from the claimed invention, in that Larson relates to a method of extracting various active ingredients from neem seed, diluting the resultant extract to form an aqueous emulsion, and then adjusting its pH to a specific range. Contrary to the Examiner's understanding, water is present and used in Larson. The presence of water as a diluent in Larson is shown at column 3, line 53-63 and in claim 1. Therefore, the cited reference does not teach or suggest forming a storage stable pesticide concentrate, which contains substantially no water. Larson clearly shows

Amendment  
U.S. Patent Application No. 09/536,351

a formulation that contains water, which is different from the claims of the present application.

Therefore, the Larson patent does not teach or suggest the claimed invention.

Third, insofar as the Dimetry et al. reference, which is cited by the Examiner to show the use of sesame oil, the Examiner has not explained how the teachings of Dimetry et al. could be combined with the teachings of Larson in order to generate the claimed invention. As explained above, Dimetry forms a formulation using water with sesame oil. Thus, even if Dimetry was combinable with Larson, a formulation formed with water would occur. This is different from the claimed invention as explained earlier. Also, Dimetry et al. uses a very small amount of sesame oil - below 1 wt% - this is quite different from the amounts recited in claims 10, 23, and 24. The remaining references would not solve these problems since none show the presence of vegetable oil in the absence of water. Larson describes an aqueous emulsion with a specific pH. Emulsions are generally unstable over long periods of time. The Examiner has not explained why one skilled in the art would assume that the addition of the another organic compound, particularly an oil, would not affect the long term stability of the emulsion, nor has the Examiner explained why one skilled in the art would believe that sesame oil would be stable in the presence of low pH solutions demanded by Larson. Accordingly, in light of all the above, this rejection should be withdrawn.

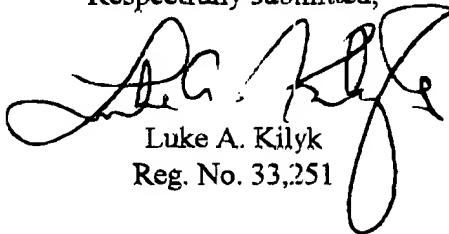
## CONCLUSION

In view of the foregoing remarks, applicants respectfully request the reconsideration of this application and the timely allowance of all the pending claims.

Amendment  
U.S. Patent Application No. 09/536,351

If there are any other fees due in connection with the filing of this response, please charge the fees to Deposit Account No. 50-0925. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such extension is requested and should also be charged to said Deposit Account.

Respectfully submitted,



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Amendment  
U.S. Patent Application No. 09/536,351

**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

1. (Amended) A pesticide formulation comprising at least one vegetable oil, at least one surfactant, and azadirachtin, wherein said formulation is storage stable and contains substantially no water.

3. (Amended) A storage stable pesticide formulation comprising at least one vegetable oil, at least one non-ionic surfactant, and a neem seed extract, wherein said neem seed extract comprises azadirachtin, and wherein said formulation contains substantially no water.

6. (Amended) The formulation of claim 3, wherein said azadirachtin is present in said formulation in an amount of from about 0.1 to about 5.0 weight %, based on the weight of the pesticide formulation.

7. (Amended) The formulation of claim 3, wherein said azadirachtin is present in said formulation in an amount of from about 0.1 to about 1.0 weight %, based on the weight of the pesticide formulation.

10. (Amended) The formulation of claim 3, wherein said formulation comprises:  
from about 1% to about 60% by weight of vegetable oil.  
from about 1% to about 5% by weight [azadirachtin] azadirachtin;  
and from about 20% to about 55% by weight of said non-ionic surfactant, based on the weight of the pesticide formulation.

14. (Amended) A storage stable pesticide formulation formed by mixing at least one vegetable oil, at least one non-ionic surfactant, and at least one neem extract together to form said formulation, wherein said neem extract comprises at least azadirachtin, and wherein said formulation contains substantially no water.

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NOTE

## Stability of Azadirachtin-A in different Organic Solvents and Aqueous Solution

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Azadirachtin-A has been found to be more stable in acetone and methanol than in chloroform, methylene chloride, carbon-tetrachloride, ethanol and water at  $29 \pm 1^\circ\text{C}$  upto 25 days. In ethanol and water, only 15.6 and 15.0% of azadirachtin-A, respectively, was recovered after 25d of incubation.

**KEY WORDS:** Azadirachtin-A, solvents, stability

The Indian neem tree (*Azadirachta indica* A. Juss) has attracted the attention of many chemists and biologists all over the world over the past two decades, because of the presence of a plethora of biologically active constituents. Azadirachtin-A is one of the most interesting constituents of neem because of its influence on insect's feeding behaviour and development<sup>1,2</sup>. It is a very sensitive molecule and can undergo changes in the presence of light, temperature, in aqueous buffer<sup>3</sup> and in organic solvents. Zanno *et al.*<sup>4</sup> (1975) reported 30% decomposition of azadirachtin-A, when kept in <sup>13</sup>C-NMR tubes in  $\text{CDCl}_3$  solution for 3 days. As there is not much information available on the stability of azadirachtin-A in other organic solvents and in aqueous solution, a systematic study has been carried out on its stability in polar and non-polar organic solvents and in aqueous solution.

### MATERIALS AND METHODS

**Materials :** Azadirachtin-A concentrate (25%) was prepared from neem seed kernel<sup>5</sup>.

Azadirachtin-A (85%) was isolated from the above azadirachtin-A concentrate by repeated column chromatography over silica-gel (60-120 mesh, eluting with *n*-hexane and ethylacetate in different ratios). It was further purified by crystallisation from carbon-tetrachloride (90%), m.p. 160° C. Its <sup>1</sup>H-NMR was in full agreement with the literature spectrum. All the solvents used in this study were of analytical grade, dried and distilled before use.

**Stability in solvents :** Stability of azadirachtin-A was studied in methanol, acetone, ethanol (95%), carbon tetrachloride, methylene chloride, chloroform and water. Distilled water used for this study was filtered through Whatman No. 42 filter paper before use to remove suspended particles. Azadirachtin-A was dissolved in above mentioned solvents @ 1mg  $\text{ml}^{-1}$  and stored in a BOD incubator for one month at  $29 \pm 1^\circ\text{C}$  in triplicate. Samples were drawn at 0, 1, 3, 5, 7, 10, 15, 20, 25 and 30 d and analysed by HPLC. The whole experiment was repeated four times.

## Stability of azadirachtin-A in different organic solvents

91

**Chromatography :** Samples were analysed by high performance liquid chromatography on a Thermo Separation Product model Spectra System P2000 liquid chromatograph, equipped with a variable wavelength UV-150 UV-VIS detector and a Rheodyne injector (20  $\mu$ l loop), connected to an Datajet reporting integrator. A Lichrosorb<sup>R</sup> RP-18 column (250 mm x 4 mm i.d.) was used. The mobile phase used was methanol-water (60:40, v/v) at a flow rate of 1.0 ml min<sup>-1</sup> and the detector was fixed at 217nm wavelength. Each sample was run for 15 minutes.

## RESULTS AND DISCUSSION

## Stability of azadirachtin - A

Per cent recovery of azadirachtin-A from different solvents at different time intervals is reported in Table 1. After 10 d of storage, the recovery of azadirachtin-A in methanol and acetone was found to be 80.0 and 85.0%

respectively, whereas in all other solvents and in aqueous solution, the recovery was less than 60 per cent (Table 1). Degradation of azadirachtin-A in different solvents followed the order methanol < acetone < carbon tetrachloride < methylenechloride < chloroform < ethanol < water. After 25 d of storage, more than 50% of azadirachtin-A was recovered from methanol and acetone solutions, whereas less than 25% was recovered from all other solvents. Per cent recovery of azadirachtin-A from ethanol (95%) and water after 25 d of storage was found to be 15.6 and 15.0 respectively. This indicated that more degradation of azadirachtin-A in ethanol than in methanol was due to the presence of water (5%). The per cent degradation of azadirachtin-A in methanol was 52.8 after 25 d of storage.

The above study indicates that for making a stable formulation, it is essential to choose the solvent in which it is stable for a long time.

Table 1. Per cent recovery of azadirachtin-A in different solvents at 29±1°C

Day	Per cent recovery*						
	Methanol	Ethanol	Methylene chloride	Carbon tetrachloride	Chloroform	Acetone	Water
0	100	100	100	100	100	100	100
1	96.8	93.7	91	94	97.2	95	94
5	92.3	90.2	82	90.8	88.9	93	88.6
7	88.3	71.6	75.4	77.2	75.3	87.8	70.5
10	80.0	58.3	60.3	62.3	47.0	85.05	55.1
15	74.4	36.2	42.8	43.3	35.7	72.2	30.4
20	59.8	28.5	30.31	35.1	30.3	53.7	28.5
25	52.8	15.6	23.12	25.8	20.28	50.8	15.0

\* Average of six replicates

## REFERENCES

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92

P. Dureja, Aditi Sinha, R.S. Tanwar and S.S. Tomar

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